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Asymmetrical local adaptation of maize landraces along an altitudinal gradient

Kristin Mercer,[1] Ángel Martínez-Vásquez[2] and Hugo R. Perales[2,3]

1 Department of Evolution, Ecology, and Organismal Biology, The Ohio State University, Columbus, OH, USA
2 Departamento de Agroecología, El Colegio de la Frontera Sur (Ecosur), San Cristóbal de Las Casas, Chiapas, México
3 Diversity for Livelihoods Programme, Bioversity International, Maccarese, Rome, Italy

Abstract

Crop landraces are managed populations that evolve in response to gene flow and selection. Cross-pollination among fields, seed sharing by farmers, and selection by management and environmental conditions play roles in shaping crop characteristics. We used common gardens to explore the local adaptation of maize (*Zea mays* ssp. *mays*) landrace populations from Chiapas, Mexico to altitude. We sowed seeds of 21 populations from three altitudinal ranges in two common gardens and measured two characteristics that estimate fitness: likelihood of producing good quality seed and the total mass of good quality seed per plant. The probability of lowland plants producing good quality seed was invariably high regardless of garden, while highland landraces were especially sensitive to altitude. Their likelihood of producing good seed quadrupled in the highland site. The mass of good quality seed showed a different pattern, with lowland landraces producing 25% less seed mass than the other types at high elevations. Combining these two measures of fitness revealed that the highland landraces were clearly adapted to highland sites, while lowland and midland landraces appear more adapted to the midland site. We discuss this asymmetry in local adaptation in light of climate change and *in situ* conservation of crop genetic resources.

Introduction

Maize, originally domesticated in central Mexico up to 9000 years ago (Matsuoka et al. 2002), is grown globally as open-pollinated landraces, or populations of traditional varieties (Aquino et al. 2001). More than 75% of Mexico's maize farmers still sow their own seeds of landrace populations rather than, or in addition to, improved varieties (Aquino et al. 2001). Farmers are key actors in the conservation of this genetic diversity because it is under their management that landraces continue to evolve. Farmers mediate the evolution of landraces by trading and mixing seed lots, by imposing selection on the populations through management practices, and by choosing seed with desirable ear and grain characteristics (Cleveland and Soleri 2007). However, natural evolutionary processes also act on these populations. Natural selection by biotic and abiotic conditions and

cross-pollination between adjoining plots are common. Repeated seed selection within a given environment could contribute to local adaptation of maize populations. Yet gene flow between landrace populations and selection by the growers and the environment for adaptive characteristics that increase fitness can play, at times, contradictory roles (Slatkin 1987; Lenormand 2002).

The result of this historic and ongoing evolution is the array of maize diversity found throughout Mexico and much of the world. More than 59 races, or phenotypic divisions, have been described in Mexico alone (Wellhausen et al. 1952; Goodman and Brown 1988; Sanchez et al. 2000). Analyses of phenotypic, molecular genetic, and cytogenetic data have helped to differentiate these races and explore their relationships to one another (Doebley et al. 1985; Bretting et al. 1990; Sanchez et al. 2000; Reif et al. 2006). The grouping of Mexican maize races through isozyme analysis produced clusters of races that

occupy similar environmental conditions or geographic regions, such as the Central and Northern Highlands cluster and the Medium to Low Elevation cluster (Sanchez et al. 2000). Some data even indicate that enzymatic diversity of maize landraces correlates well with altitude (see Fig. 4 in Doebley et al. 1985). Therefore, there are genetic relationships among races and some of the genetic divergence may be attributed to variability in environmental tolerances. As a result, some authors assume that strong local adaptation has occurred in maize landraces (Cleveland et al. 1994; but see Wood and Lenne 1997). Some claim that there are 'locally adapted landraces for every valley' (Eagles and Lothrop 1994, p. 14). Yet actual patterns of local adaptation in maize are not well studied. While there can be significant variation among populations within a given race (Herrera-Cabrera et al. 2004; Pressoir and Berthaud 2004; Reif et al. 2006), whether it is concordant with local adaptation has not been addressed from an evolutionary perspective.

Previous studies of local adaptation in crop species have revolved around identifying genetic variability that could be useful for modern plant breeding efforts. Plant breeders have long desired to produce broadly adapted, improved varieties for small, subsistence landholders in marginal environments as a way to increase production (Evans 1993 p. 164; Braun et al. 1996; Jiang et al. 1999). Highland regions of the world are one such marginal environment. In this endeavor, breeders have mixed 'adapted' highland and higher yielding lowland germplasm to explore the genetic possibilities and constraints of producing varieties with broader environmental tolerances (Eagles and Lothrop 1994). Through this work we can gain an understanding of general responses of various highland and lowland breeding populations to various environments. For instance, a highland hybrid in Kenya was found to have the highest yield at the highland site and lowest yield at the lowland site (Cooper 1979). In a similar study in Mexico, Lafitte and Edmeades (1997) found that highland breeding populations did poorly in lowland areas and lowland breeding populations did poorly in the highlands. These differences could be the result of morphological, phenological and physiological differences between types. Indeed, a review of breeding populations derived from races of central Mexico reveals differences between highland and lowland types in photosynthetic rate, growth rate, timing of seedling emergence and flowering, stem color, and tolerance to frost, cold, drought and hail (Eagles and Lothrop 1994).

These studies focus more on information that will facilitate the production of broadly adapted varieties and less on the evolutionary ecology of maize populations. Nevertheless, they provide intriguing patterns on which to build a more fundamental understanding of regional maize evolution. We aim to understand not just the general responses of altitudinal groupings of populations to new environments, but also the population-specific responses. This will clarify how genetic variation is distributed within and among maize races across the landscape. By studying these patterns and their relationship to fitness, we will explore how maize landraces interact with and evolve in response to the environment.

In the context of global climate change, local adaptation of landraces is especially important to understand. Models for southern Mexico suggest a 3°C increase in temperature and a 10–15% reduction in annual precipitation over the first half of the 21st century (Christensen et al. 2007). In the face of these changes, we can expect a range of responses in natural and agricultural plant populations: evolutionary adaptation to the novel conditions (Franks et al. 2007); adaptively plastic responses that allow for maintained productivity; migration into areas with more optimal conditions; or extinction. Given that anthropogenically managed crop populations cannot migrate on their own and given the conservation value of diverse maize varieties, climate change requires us to ask, will maize populations evolve and/or express plasticity to handle the new conditions? Will certain types of maize or specific areas of production respond more negatively to climate change? How could climate change affect genetic conservation of certain maize races?

In this article we offer preliminary insights into the ways that natural selection may have played a role in organizing the present maize diversity in Chiapas, Mexico. We chose to further explore adaptation of maize landraces to altitude, a factor that covaries with temperature and moisture gradients. In particular, lower elevation sites have environmental conditions similar to those predicted for the highlands in the future, allowing us to explore how landraces may respond as climate changes. To do so we established common garden experiments in the midland and highlands of Chiapas and assessed the fitness of maize collected from low-, mid-, and high-elevations. We address two main questions: (i) are landrace populations from different altitudes locally adapted to their home altitude? (ii) is there variability among populations from a given altitudinal range in their response to distinct environments?

Methods

Study location

Chiapas, Mexico is an optimal place to study local adaptation because maize is grown from 0 to 2600 m and under a range of soil, radiation, temperature and precipitation conditions. Twenty-two races of maize have been collected in the state, making it second only to Oaxaca in

racial diversity (Perales and Hernandez-Casillas 2005). Altitude is an important factor correlated with this diversity, with certain races found primarily in particular altitudinal ranges (See Table 2 in Brush and Perales 2007).

Maize populations

From 2000 to 2004, a large collection of maize landraces was undertaken throughout Chiapas, Mexico, comprising more than 3000 samples (see Brush and Perales 2007 for description). The populations had been classified into races based on ear morphology following Wellhausen et al. (1952) and were typical of races found at those elevational ranges (see Table 2 in Brush and Perales 2007). Twenty-one of these populations were selected for our study, each made up of bulked seed from 50 ears produced in 2004. The seeds used in this experiment were those collected from the farmers.

To match the altitude of our common gardens (see below) with that of our maize populations, we included landrace populations that had been collected at an altitude approximately ±100 m from that of the common garden sites (see Table 1 for description of collections and Fig. 1 for their locations). Thus, we had seven landraces from 690 to 880 m (lowland), seven from 1530 to 1670 m (midland), and seven from 2170 to 2340 m (highland). These ranges correspond to commonly understood altitudinal divisions for the lowlands (1–1200 m), midlands (1200–2000 m), and highlands (>2000 m). Because of the distribution of landraces on the landscape, all highland populations collected were from one race

(Oloton), midland populations were from two races (Comiteco, Olotillo), and lowland populations from four races (Comiteco, Tuxpeno, Cubano, Zapalote). Populations designated with a and b of the same number were collected from different farmers in the same community (Table 1). In some cases the two populations are from different races and they usually have different grain colors (Table 1).

The communities sampled, which were made up of primarily mestizo or indigenous families, were relatively small with 50–500 households per village (INEGI 2001). In almost all cases, farmers had originally acquired their seed within their community through family members or acquaintances. However, one seed lot (population 2b) had been obtained from another community in Chiapas and two farmers in southern Chiapas stated that their seed was originally introduced from Guatemala (populations 10b and 14b; Table 1). There was considerable variability in the number of years farmers had used their seed lot, ranging from 2 to >40 years and with a median of 7 years. However, seed could have been in the community for far longer (H. R. Perales, unpublished data).

Common gardens

In 2005, one common garden was established at each of three altitudes within Chiapas, Mexico: Ozozocoautla at 700 m (lowland), Comitán at 1500 m (midland), and San Cristóbal de Las Casas at 2150 m (highland). Because of logistical problems at Ozozocoautla, only data from the latter two common gardens are discussed here. San

Table 1. Description of 15 maize locations where 21 landrace populations were collected in Chiapas, Mexico. See Fig. 2 for map.

Altitudinal grouping	Collection location ID	Municipality	Community	Latitude (N)	Longitude (W)	Altitude (m)	Maize population ID	Maize race	Grain color
Lowland	1	Cintalapa	Villa Morelos	16°28′44″	93°35′37″	690	1	Zap	W
	2	Ocozocuautla	San Isidro El Gavilan	16°45′49″	93°27′21″	730	2a, 2b	Tux,Tux	W,W
	3	Tzimol	Manuel Velasco Suarez	16°07′44″	92°14′00″	690	3	Cub	Y
	4	La Trinitaria	Chihuahua	16°01′48″	91°58′24″	880	4a, 4b	Com,Tux	Y,W
	5	La Trinitaria	Las Delicias	15°58′08″	91°51′45″	840	5	Cub	Y
Mid-elevation	6	Jitotol	Jitotol	17°04′02″	92°51′39″	1630	6	Com	Y
	7	Zincantán	La Granadilla	16°42′39″	92°50′39″	1560	7	Com	W
	8	Venustiano Carranza	Aguacatenango	16°28′20″	92°24′28″	1670	8a, 8b	Olo,Com	W,Y
	9	Comitán	San Francisco El Rincón	16°17′05″	92°08′05″	1590	9	Com	W
	10	Siltepec	Siltepec	15°33′25″	92°19′21″	1530	10a, 10b	Com,Com	Y,W
Highland	11	Chamula	Los Ranchos	16°49′46″	92°42′01″	2200	11	Oln	Y
	12	Zincantán	Nachij	16°43′43″	92°43′38″	2170	12	Oln	Y
	13	San Cristóbal	Ej. Pedernal	16°39′50″	92°27′18″	2180	13	Oln	Y
	14	La Grandeza	Llano Grande	15°28′22″	92°13′54″	2340	14a, 14b	Oln,Oln	W,Y
	15	Motozintla	Buenos Aires	15°19′56″	92°16′10″	2240	15a, 15b	Oln,Oln	W,Y

Com = Comiteco, Cub = Cubano Amarillo, Oln = Oloton, Olo = Olotillo, Tux = Tuxpeno, Zap = Zapalote; Race following Wellhausen et al. (1952). Y = yellow, W = white.
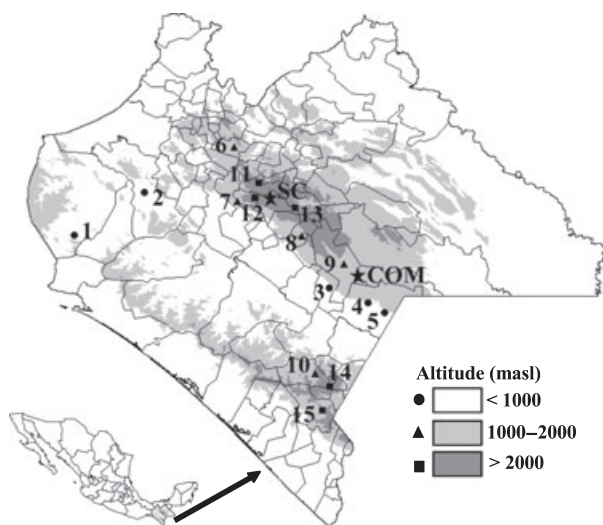
Figure 1 Map of Chiapas, México with altitude. Numbers mark the origins of maize populations (some locations are the origin for more than one population) collected in 2004. Lowland locations (∼800 m) are indicated with circles, the midland locations (∼1400 m) with triangles, and the highland locations (∼2200 m) with squares. The locations for the San Cristóbal de Las Casas (SC) and Comitán (COM) common gardens established in 2005 are marked with stars.

Cristobal has an average of 1100 mm of precipitation a year and an average minimum and maximum temperature of 8.1–21.8°C (annual mean of 15.0°C). Precipitation is similar at Comitan (averages 1050 mm), but the average minimum and maximum temperatures are higher (12.8–25.4°C with an annual mean of 19.1°C). At San Cristobal frosts between November and March can affect early or late maize plantings; Comitan is completely frost-free. The conditions at the midland site produce more potential for evapotranspiration during the season than those at the highland site. Due to seasonal differences at the two sites, we planted in early May in San Cristóbal and mid-July in Comitán; these dates were within the typical range for each location.

Into each of these gardens, we planted seed from all 21 of our maize populations in a complete randomized block design. Maize plants from an individual population were planted into 4.8 × 3.2 m plots, which were replicated three times within each site. Plots contained four rows (0.8 m apart), seven *matas* (or planting positions) per row (also 0.8 m apart), and two plants per *mata*. The 48 plants per plot approximated a target density of 31 250 plants per ha, similar to that used locally by farmers. Plant density was standardized by oversowing and thinning.

Data collection

To reduce edge effects, border plants were excluded and data were taken on only 10 plants in the center of the

plot. In the field, we recorded data on flowering phenology and plant morphology, and collected all ears produced by each plant. Subsequently, we determined whether or not the plant had produced seed (i.e., if it had an ear with at least one seed) and whether the ears produced were good quality (i.e., if the ear had <50% rotten seed and would therefore be judged by a farmer to be worth selecting for the following year's seed). Then we weighed the dry mass of the seeds from the good quality ears and noted morphological characteristics of the ear (H. R. Perales, unpublished data).

To characterize the fitness of landraces that originated from the different elevation zones, we used three fitness components. The first, the proportion of plants that produced good quality seed, was measured at the scale of the plot. Plants that produced no seed at all, or that produced only rotten seed, were distinguished from those that produced good quality seed. The second fitness measure was the average mass of good quality seed (in grams) per plant for those that produced good quality seed for each plot. The plot level averages of these two metrics were then multiplied together to produce a more integrative fitness measure, adjusted fitness, which weighed good quality seed production per plant by the likelihood of producing good quality seed [good quality seed mass per plant (g) × probability of producing good quality seed = adjusted fitness (g)].

Analyses

Data on the probability of producing good quality seed, total mass of good quality seed per plant, and adjusted fitness were analyzed in two ways for each trait. In the overall analysis, all data were analyzed together to enable us to assess interactions between maize altitudinal groups and common garden location (i.e., altitudinal group by common garden G × E). Secondly, we separated the data by altitudinal grouping and investigated the responses of individual maize populations within each group to the distinct environmental conditions (i.e., population within altitudinal grouping by common garden G × E). Two pairs of populations within each altitudinal grouping were collected from different farmers in the same community (e.g., 2a and 2b). Because of their different phenotypes and responses to the environment, we have treated them as independent.

In both sets of analysis, we used plot nested within population by altitudinal group by common garden as an error term. Using SAS Mixed to perform ANOVAs, we calculated least squares means values and standard errors, and made Tukey–Kramer adjusted comparisons. Transformations of the data improved the distribution of seed mass, but did not affect the statistical results enough to

warrant transformation. Relative fitness at a given common garden was calculated as the adjusted fitness of an altitudinal grouping divided by the adjusted fitness of the local altitudinal grouping. For instance, in the highlands the relative fitness of the midland landraces was their adjusted fitness under highland conditions divided by the adjusted fitness of highland landraces under highland conditions.

In this study, local adaptation was found when there was a significant interaction between genotypes and the environmental conditions such that local types had higher adjusted fitness than nonlocal types (Kawecki and Ebert 2004). Other studies have used a one degree of freedom test to see if the local type outperforms the nonlocal types in all locations simultaneously (Thrall et al. 2002).

Results

Responses of altitudinal groupings to common gardens

The three maize altitudinal groupings responded differentially to the common garden environments with crossing reaction norms for the proportion of plants that produced good quality seed. This G × E interaction could be seen in the significant common garden by altitudinal grouping effect (Table 2A). Lowland landraces had the greatest proportion of plants that produced good quality

seed regardless of environment (∼88%; Fig. 2A). Like the lowland landraces, the probability that midland landraces produced good quality seed was not strongly affected by environment (57% and 68% in the midland and highland environment, respectively), though the values were lower (Fig. 2A). By contrast, the probability of producing good quality seed for highland landraces was strongly affected by the environment, with very few plants producing seed in the midland garden (∼24%) and many more producing seed in the highland garden (∼83%; Fig. 2A). Overall, we found greater variation among altitudinal groups at the midland site than in the highland site (Fig. 2A).

In addition to the significant G × E interaction (Table 2B), differences among altitudinal groupings in mass of good quality seed per plant were particularly clear in the highlands where both highland and midland landraces outperformed the lowland landraces (∼164 vs 122 g; Fig. 2B). Differences were smaller at the midland site and the order of the landraces changed, with the highest production per plant for the midland landraces (∼67 g) and the lowest production for the highland landraces (∼52 g; Fig. 2B).

When we combined the two types of data to analyze adjusted fitness, we found that the crossing norms of reaction for the more comprehensive fitness measure were indicative of G × E interactions (Table 2C). In the

Table 2. ANOVA results for probability of producing selectable seed, mass of selectable seed produced, and adjusted fitness.

Effect	Overall			Lowland populations			Midland populations			Highland populations		
	DF†	F	Signif.	DF	F	Signif.	DF	F	Signif.	DF	F	Signif.
A. Probability of producing selectable seed												
Common garden	1, 80	97.16	****	1, 24	0.01	ns	1, 24	9.47	**	1, 24	131.67	****
Replication	4, 80	1.32	ns	4, 24	0.47	ns	4, 24	0.9	ns	4, 24	0.72	ns
Altitudinal grouping	2, 80	73.73	****	–	–	–	–	–	–	–	–	–
CG*Alt	2, 80	57.47	****	–	–	–	–	–	–	–	–	–
CG*Population (Alt)	36, 80	8.65	****	12, 24	0.46	ns	12, 24	28.7	****	12, 24	2.88	**
B. Weight of selectable seed (g)												
Common garden	1, 71	339.02	****	1, 24	226.5	****	1, 19	91.8	****	1, 20	76.7	****
Replication	4, 71	3.84	**	4, 24	5.33	**	4, 19	1.61	ns	4, 20	0.38	ns
Altitudinal grouping	2, 71	11.35	****	–	–	–	–	–	–	–	–	–
CG*Alt	2, 71	11.26	****	–	–	–	–	–	–	–	–	–
CG*Population (Alt)	32, 71	2.44	***	12, 24	4.19	***	10, 19	0.94	ns	10, 20	2.85	*
C. Adjusted fitness (g)												
Common garden	1, 80	706.99	****	1, 24	95.39	****	1, 24	110	****	1, 24	1188.8	****
Replication	4, 80	0.67	ns	4, 24	1.59	ns	4, 24	0.22	ns	4, 24	0.38	ns
Altitudinal grouping	2, 80	1.15	ns	–	–	–	–	–	–	–	–	–
CG*Alt	2, 80	45.02	****	–	–	–	–	–	–	–	–	–
CG*Population (Alt)	36, 80	10.9	****	12, 24	1.76	ns	12, 24	14.33	****	12, 24	16.56	****

Separate analyses were run with PROC MIXED: overall and by altitudinal grouping of maize populations. Data were collected in 2004 from maize plants grown in a highland garden in San Cristobal de Las Casas and a midland garden in Comitan, Chiapas, Mexico.
*<0.05, **<0.01, ***<0.001, ****<0.0001; †numerator df, denominator df.
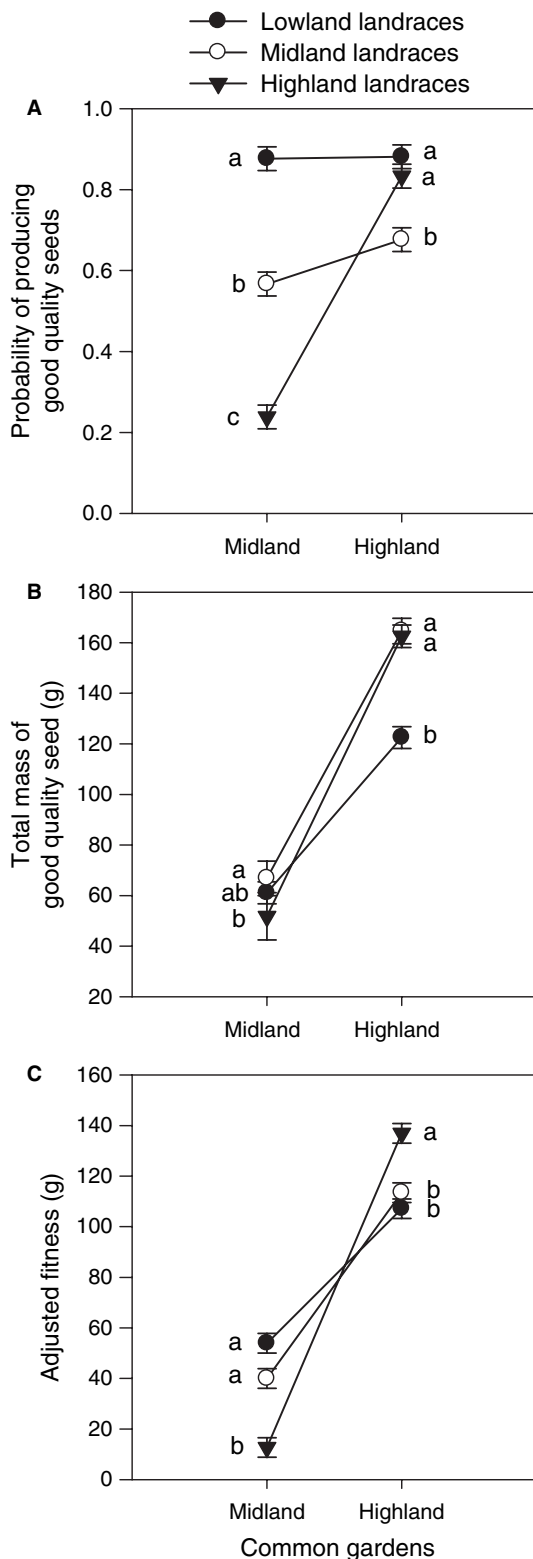
Figure 2 Fitness responses of lowland, midland and highland maize landraces to midland and highland common garden conditions in Chiapas, México, 2005. Panels present least squares means, standard error bars, and Tukey–Kramer adjusted contrasts of (A) probability of producing good quality seed, (B) total mass of good quality seed produced per plant, and (C) the adjusted fitness (a × b).

under highland conditions, the highland landraces had significantly higher fitness than the mid- or lowland landraces. Clearly, the highland landraces were at a great disadvantage outside their usual altitude (Fig. 2C). At the midland site, the fitness of the highland landraces relative to the local midland landraces was 33%, while that of the lowland landrace was 141%. By contrast, in the highlands, the fitness of midland and lowland landraces relative to the local highland landraces was 80–82%.

Responses of individual populations to varied conditions

Separate analyses of the probability of producing good quality seeds for each population within its altitudinal grouping revealed interesting contrasts (Fig. 3). The probability of producing good quality seeds was uniformly high for lowland populations, ranging from 0.80–0.93 to 0.80–0.97 (Fig. 3A) in the midland and highland sites, respectively (Table 2A). The midland populations responded differently. The probabilities of producing good quality seed ranging from 0 to 0.967 in both locations and norms of reaction differed among populations (Fig. 3B). This was reflected in the significant G × E interaction (Table 2A). Two midland populations in particular (populations 7 and 10a) had much less success than the others, especially in the midland site (Fig. 3B). For highland populations the probability of producing good quality seed varied widely when grown in the midlands, but was generally low (0.033–0.50; Fig. 3C). The variability among populations was lower in the highlands, ranging from 0.67–0.93 (Fig. 3C). Again, we found a significant G × E interaction (Table 2A), but this interaction became nonsignificant when the data were transformed to their square, so it must be interpreted with caution. Within the lowland and highland altitudinal groupings we saw significant G × E interactions for mass of good quality seed per plant (Table 2B; Fig. 3D–F).

Although there were some population-specific adjusted fitness responses to the two common gardens for the highland and lowland groupings, the greatest variability in response was among midland populations (Table 2C; Fig. 3G–I). Under midland conditions, two populations had an adjusted fitness of zero, while the other five clustered together (Fig. 3H). In this way, two populations responded more like highland populations, while the others responded similarly to the lowland populations.
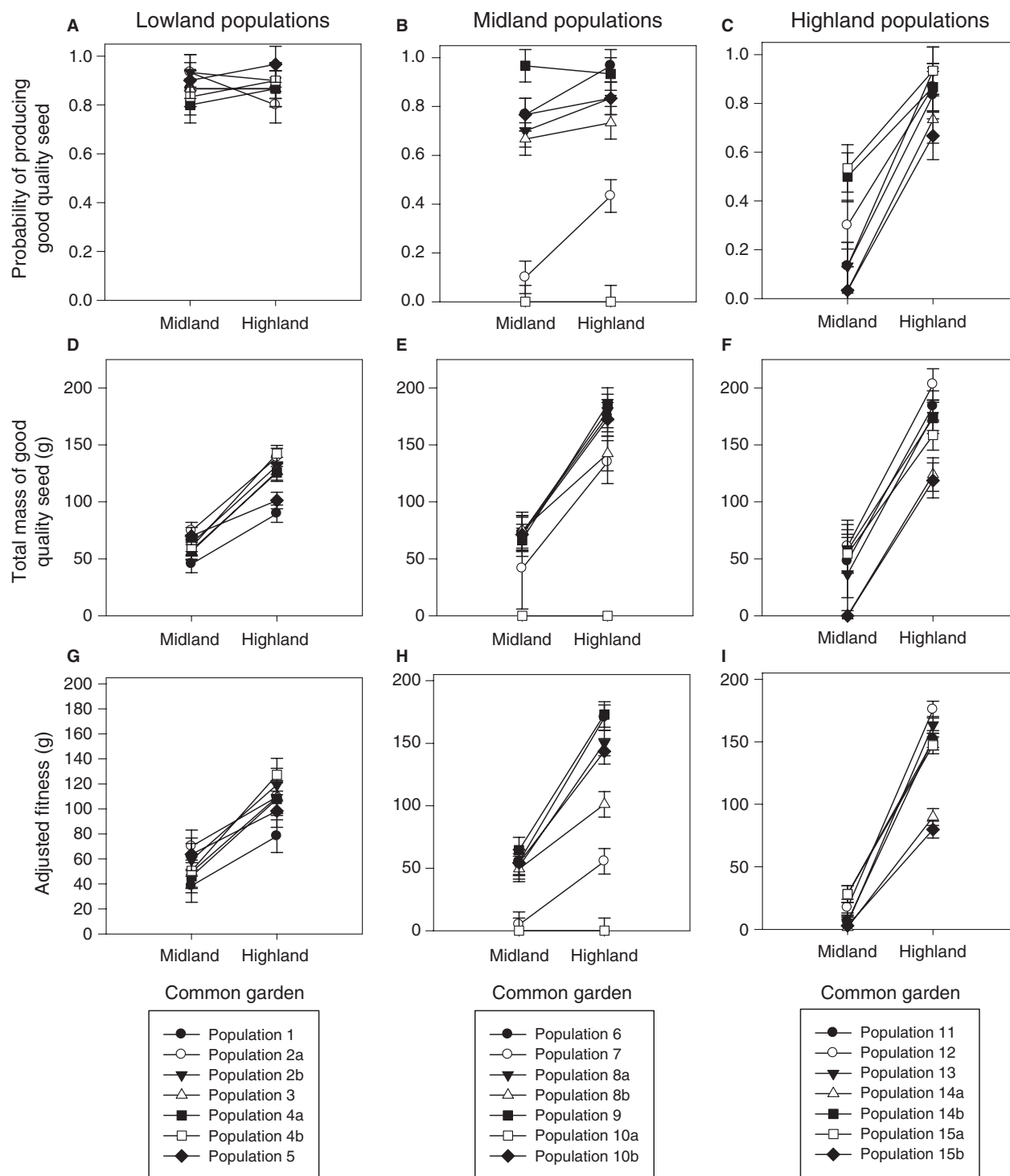
midland site, midland and lowland landraces had higher fitness than the highland landraces, with those from the lowlands doing marginally best (Fig. 2C). By contrast,

Figure 3 Fitness components for lowland, midland, and highland maize populations grown in midland and highland common gardens in Chiapas, Mexico, 2005. We present least squares means with standard error bars for the probability of producing good quality seed for populations from the (A) lowlands, (B) midlands, and (C) highlands; the total mass of good quality seed in grams (excluding zeros) for populations from the (D) lowlands, (E) midlands, and (F) highlands; and the adjusted fitness in grams for populations from the (G) lowlands, (H) midlands, and (I) highlands.

Under highland conditions the variability in response broadened. One population still had zero fitness, but the adjusted fitness of the others was highly variable ($\sim$55–173 g; Fig. 3H).

Discussion

These data provide evidence for differing degrees of local adaptation of maize landraces to their elevation of origin. Local adaptation is identifiable when there is a significant interaction between genotypes and environmental conditions, such that the local type outperforms other types in its own environment (Kawecki and Ebert 2004). This was certainly the case for the highland and midland maize when considered alone. The adjusted fitness of the highland landraces was greater than that of the midland landraces under highland conditions. In the midlands, the adjusted fitness of the midland landraces was greater than that of the highland landraces. However, the inclusion of our lowland maize complicated the story. While the midland landraces did outperform the highland landraces by a large margin in the midlands, the lowland maize was as fit as the midland maize (this was marginally nonsignificant). Lafitte and Edmeades (1997) saw a similar pattern, with lowland breeding populations of maize outperforming the midland breeding populations at midland locations, although they could not mechanistically explain these differences.

By combining two fitness components we were better able to understand how maize fitness responded to varied environments. When we consider adjusted fitness under midland conditions, lowland landraces performed best and highland landraces performed worst. The greater fitness in the lowland landraces derived primarily from the greater percentage of their plants producing good quality seed. Midland plants that produced good quality seed tended to produce more of it in the midlands than lowland plants did. The weak adjusted fitness of the highland maize in the midland site was strongly related to the low proportion of plants producing good quality seed under those conditions. Conversely, under highland conditions, the highland maize performed well because both fitness components were consistently high.

There could be a number of reasons for these differences in fitness. Environmental conditions in the midlands could have been outside the range that highland plants could tolerate physiologically. Unlike many mountainous systems, the higher temperatures and greater potential for evapotranspiration in the midlands at the end of the rainy season may make them comparatively more stressful than highland environments. Highland environments, by contrast, are cooler and have a longer growing season as water deficits do not limit growth so

soon after the rains cease. Highland plants may not have been able to avoid (through phenological characteristics) or tolerate (through physiological mechanisms) midland conditions, which would have ultimately reduced their fitness. This may have been manifested in the production of underdeveloped ears which could have been prone to disease. Lowland populations may have been less stressed in the midland environment than in the conditions under which they had evolved, allowing them to be relatively fit. However, their reduced fitness in the highlands may stem from an inability to fix enough carbon in the cool temperatures before flowering, thereby reducing seed production. Studies on timing of flowering, water use efficiency and other characteristics could uncover factors controlling fitness in novel environments.

From individual population data we can see that norms of reaction were not uniform across populations within altitudinal groups. The adjusted fitness of four midland and five highland populations was similar and high at the highland site. In both groups, the other populations had lower adjusted fitness values. Therefore, some high-performance midland populations matched high-performance highland populations under highland conditions, but the greater variability in performance among midland populations brought down their average. The adjusted fitness of most midland populations in the midland site was equivalent to that of the lowland populations, but there were two populations that reduced the overall mean considerably. Most midland and lowland populations appear to be similarly adapted to the midland site. Yet the greater variability among midland populations was surprising as they do not seem to come from more diverse origins or altitudes that the highland or lowland types. In fact, the lowland populations, which have the lowest diversity of fitness responses, were categorized as coming from the greatest number of races.

Differences between individual populations might be due to seed origin. The two populations collected closest to the midland and highland common garden locations (populations 9 and 12, respectively), were among the most fit in their home environment (Fig. 3G–I). By contrast, the two populations from the highlands that had the lowest fitness (populations 14a and 15b) originated in southern Chiapas near the Guatemalan border, as did the population from the midlands that had an adjusted fitness of zero (population 10a; Fig. 3G–I). It is possible that, although these populations came from similar altitudinal conditions, they could have adaptations to environmental conditions that differ from those of our experimental sites. For instance, rainfall patterns and soil types in the two mountain ranges may differ. Despite these results, the rest of the populations from these southern communities (populations 10b, 14b, and 15a)

had adjusted fitnesses similar to the other samples from the highland or midland zones (Fig. 3G–I), even though some may have originated from Guatemala (see Methods).

So would removing populations collected where conditions or cultural practices may be different than those at our common garden affect our results? We explored this through two reanalyses of adjusted fitness. In the first, populations were removed for having been collected farthest from the common garden sites (populations 6, 7, 14 a&b and 15 a&b) to test if focusing on populations originating near the gardens, from presumably more similar environments, alters our interpretation. This reanalysis did not change our results (data not shown), indicating that the patterns of local adaptation to altitude were robust regardless of the locations where populations were collected.

In the second reanalysis, populations with consistently low adjusted fitness were removed (populations 7, 10a, 14a and 15b) as their fitness reduction could have resulted from adaptations to conditions of their home environment not found in our gardens. In this second reanalysis adjusted fitness for midland and lowland landraces became indistinguishable at the midland site (midland fitness was somewhat greater than lowland fitness) and the fitness of midland and highland landraces grown in the highlands no longer differed (highland fitness was somewhat greater than midland fitness; data not shown). However, the general interpretation was not substantively different: there were strong fitness consequences when transplanting landraces to much higher altitudes and to lower altitudes. Therefore, we confirm that altitude is an important factor driving local adaptation (Perales and Hernandez-Casillas 2005; Brush and Perales 2007). Nevertheless, we cannot rule out that some environmental factors that covary with altitude or cultural practices unique to a given region (Perales et al. 2005; Brush and Perales 2007) could also complicate the selection pressures acting on maize populations. For example, unique disease or pest pressures in one location could select for resistance that reduces fitness in other areas. Or weed control practices could differ, selecting for populations with different seed germination and competitive characteristics.

Fitness could have also varied among our populations because of maternal effects (Roach and Wulff 1987) or other transgenerational epigenetic effects. As for this experiment it was prohibitive to grow these populations in a uniform environment for one generation and make hand-pollinations to maintain population identity. The diverse environments of the farmers' fields and storage areas could have affected plant characteristics and fitness in our study. In general, both common maternal effects and epigenetic effects could theoretically cause changes in fitness that parallel local adaptation (Wendel and Wessler 2000), although to our knowledge this is not well-studied. Nor is it well understood whether producing seed of differentially adapted plants in a single garden whose environment that is not equally benign to all could differentially alter seed quality or produce effects on subsequent fitness through the interaction between maternal genotypes and the environment.

One key question remains: how would these landraces have fared under lowland conditions? We now expect, given the rest of our data, that the fitness hierarchy would have been lowland>>midland>highland. It is possible that highland plants would not have set any seed and midland landraces might have been unfit, as well. The fitness of lowland maize would have been expected to remain high. Future studies should include common gardens at more altitudes, at multiple sites per altitude, and over multiple years. In this way, altitude *per se* could be decoupled from the environmental factors that covary with it.

Asymmetry of local adaptation

The highland populations were clearly sensitive to the conditions present in the midland site and were more sensitive to transplantation than either the lowland or the midland types. Note the adjusted fitness of each type relative to the local type at each location. The relative fitnesses of ∼80% for the midland and lowland types in the highland site indicate that they did not produce as well as the highland types under those conditions, but they did produce a decent amount of seed per plant. By contrast, the highland types only produced 33% of that of the local midland types under midland conditions – an extreme disadvantage. Yet it is surprising that the lowland landraces did not suffer a similar disadvantage in the highlands.

This asymmetrical local adaptation would indicate that, in general, it is harder for highland varieties to move down in elevation than for midland and lowland varieties to move up. In other words, highland populations may possess adaptations to highland environments that become costly under midland conditions. These adaptations may involve physiological (e.g., water use efficiency), phenological (e.g., flowering time), or morphological characteristics (e.g., number of leaves). Alternatively, they may not possess characteristics that are essential in midland environments, such as disease resistance. It may also be that midland and lowland landraces express great phenotypic plasticity, which may explain their uniformly high fitness.

Studies of natural plant populations collected along environmental and altitudinal gradients and using common garden experiments have revealed many cases of

local adaptation (Heisey et al. 1942; Etterson 2004; Geber and Eckhart 2005). In these studies, phenological, morphological, and physiological factors helped explain the fitness responses of populations to translocations. For instance, Heisey et al. (1942) found that the longer time to flowering of subalpine yarrow populations obviated their ability to reproduce in alpine conditions. Etterson (2004) found that the lower water use efficiency and larger leaf size of northern populations of *Chamaecrista* reduced their fitness when grown in the south.

In future work we would like to better understand the mechanisms governing the local adaptation of maize landraces and the role of maternal effects in producing this pattern. Past studies of highland and lowland maize have shown that they differ in phenological, morphological, and physiological characteristics in ways that affect growth and yield. For instance, highland plants can grow faster as seedlings and generally advance more quickly towards tasseling under cooler conditions (Hardacre and Eagles 1989; Ellis et al. 1992; Stehli et al. 1999), although the early seedling vigor of highland plants is lost by anthesis (Stehli et al. 1999). An early study by Cooper (1979) found that the potential grain production for highland plants (measured as number of grains on embryonic cobs) was greatest at lower elevations, while actual grain production was highest at higher elevations. Similarly, Goldsworthy et al. (1974) found more 'barren plants' at lower elevation than at mid-elevations. The thermotolerance of important enzymes (Burke et al. 1988; Turner et al. 1994), as well as UV protection and responses may also differ between highland and lowland plants. It is likely that these mechanisms could be important in organizing the maize diversity in Chiapas.

Similar studies with more species in other crop centers of origin would broaden our understanding of the environmental tolerances and adaptive differences of crop landraces. Agronomists and plant breeders often take a common garden approach, using multiple locations to compare varieties in yearly yield trials. These trials allow for the ranking of improved varieties across regions. However, due to the way varieties are chosen, the use of yield rather than fitness measures, and the interpretation of the results, they do not necessarily help us to understand the evolutionary processes governing the partitioning of crop genetic diversity across the landscape, nor how that diversity would respond to novel environmental conditions. An evolutionary perspective that uses the study of natural populations as a guide while incorporating the anthropogenic aspects of crop production and management is most useful for understanding these issues.

The results from our study have important implications for the conservation of landraces in germplasm banks (*ex situ*) or in farmers' fields (*in situ*). Although *ex situ* collections of crop genetic diversity are essential for large-scale conservation of globally important germplasm, farmers have come to be seen as key actors in the conservation of crop diversity (UN 1992; IPGRI 1993; NRC 1993), especially in crop centers of origin (Altieri and Merrick 1987; Brush 1995; Bellon 1996). Maize diversity in Mexico has been reduced by the planting of improved hybrid varieties, but not in a uniform manner (Bellon and Brush 1994; Louette et al. 1997), as expected by some (Cleveland et al. 1994). Landraces have been replaced by modern varieties more in the lower altitudes than in the highlands, which have been protected from loss since few improved varieties perform as well as landraces under the environmental and agronomic conditions of the highlands (Perales et al. 2003). Yet landrace diversity may be threatened by climate change. Our data suggest that it will become more important than ever to grow out *ex situ* highland accessions in high elevations to ensure the viability and longevity of those seed collections. Similarly, highland sites should be promoted for the *in situ* conservation of highland populations to promote their evolution as climate shifts. The development of local seed saving and sharing efforts appear to be necessary in the highlands to conserve the unique genetic diversity found there. Due to the apparent variability among midland and highland populations in their responses to novel environments, more accessions may be needed to capture this potential range of responses than would be needed for lowland landraces.

These results provide preliminary information for understanding how climate change could affect subsistence maize production in Chiapas. As temperatures rise, the cool environment to which highland landraces appear to be particularly well-adapted will begin to disappear. These highland landraces appear to be very sensitive to the conditions more typical of midland sites, which include higher temperatures, altered precipitation, shorter growing seasons, and different insect and disease communities. Reductions in the ability of highland plants to produce good quality seed could reduce yields for highland subsistence farmers, potentially affecting livelihoods. Future research should clarify the mechanisms governing local adaptation in these maize populations, as well as investigate the potential for adaptive evolution to proceed in landrace populations in response to climate change.

Nevertheless, difficult questions arise here. Our preliminary results suggest that current highland varieties will not produce well as temperatures rise. That is, unless they respond to these novel selection pressures by adapting. If they adapt, will they maintain their unique characteristics, become like current midland populations, or will they evolve to be another race altogether? Midland and

lowland varieties could be considered poised to fill-in for faltering highland landraces. They produce about 80% of what highland landraces do in the highland environment. This raises the questions, how will farmers' informal seed networks adapt? Will highland farmers begin to seek out maize populations from lower elevations? What role should researchers play in facilitating change and how can they forestall reductions in diversity? Evolutionary theory and ecological research are well-placed to contribute to answering these questions.

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